

Supplemental Material to:

Yi-An Chen, Mathieu Lemire, Sanaa Choufani, Darci T. Butcher, Daria Grafodatskaya, Brent W. Zanke, Steven Gallinger, Thomas J. Hudson and Rosanna Weksberg

Discovery of cross-reactive probes and polymorphic CpGs in the Illumina Infinium HumanMethylation450 microarray

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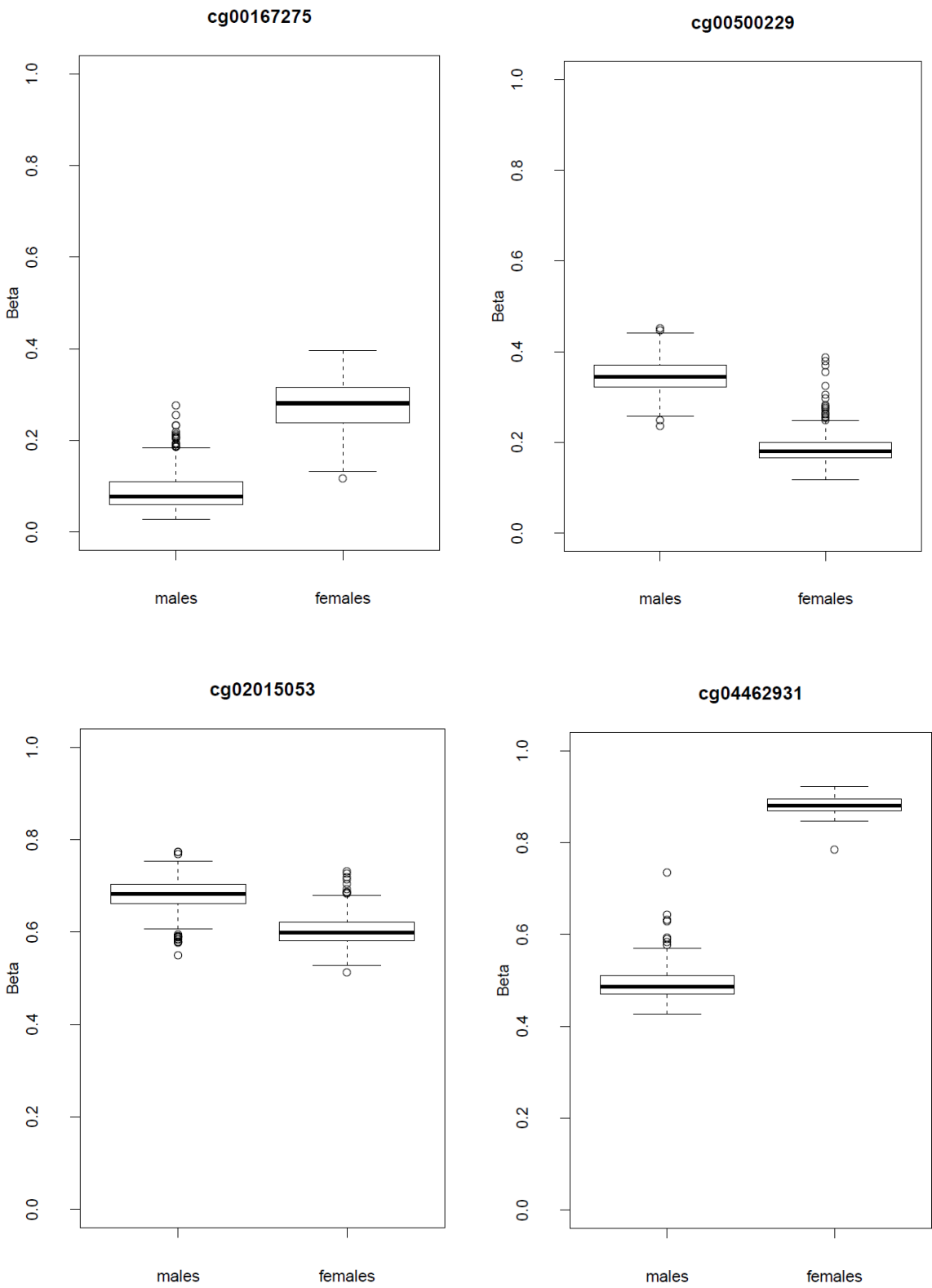
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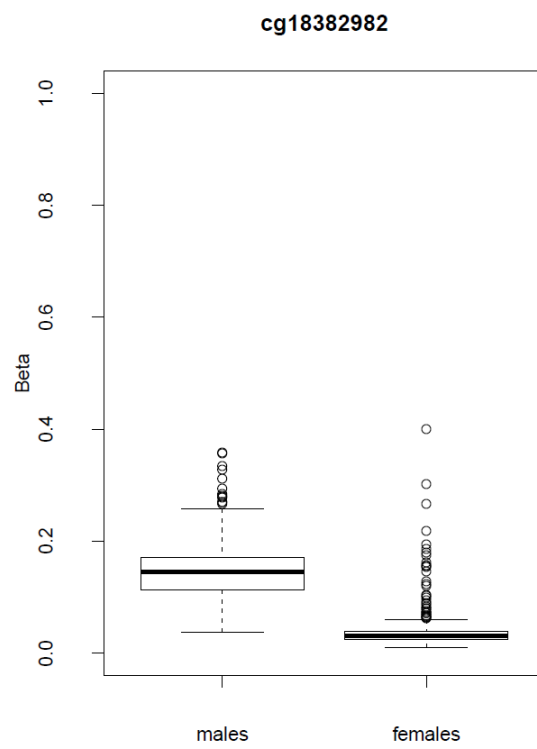
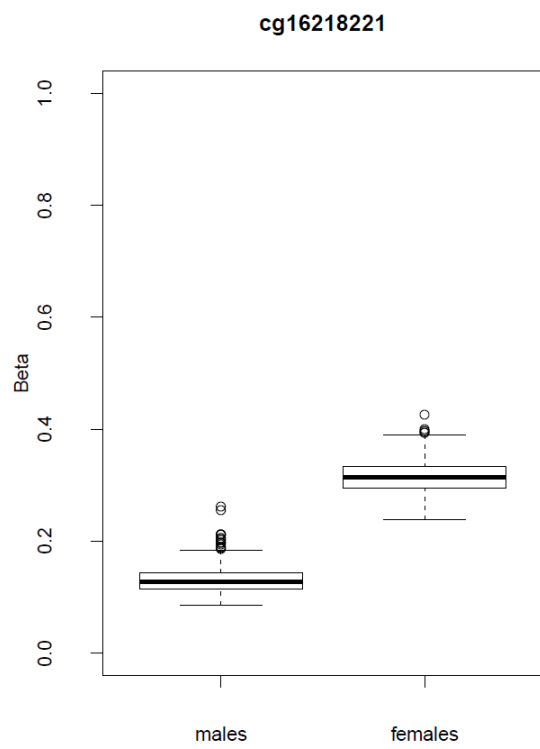
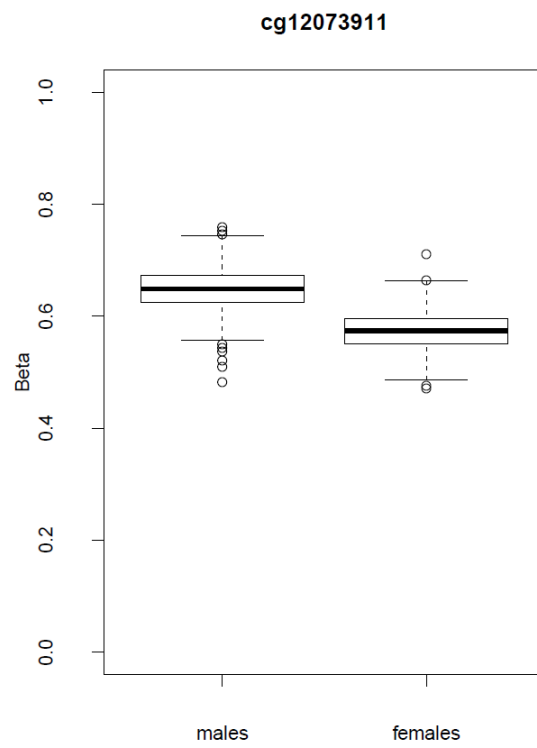
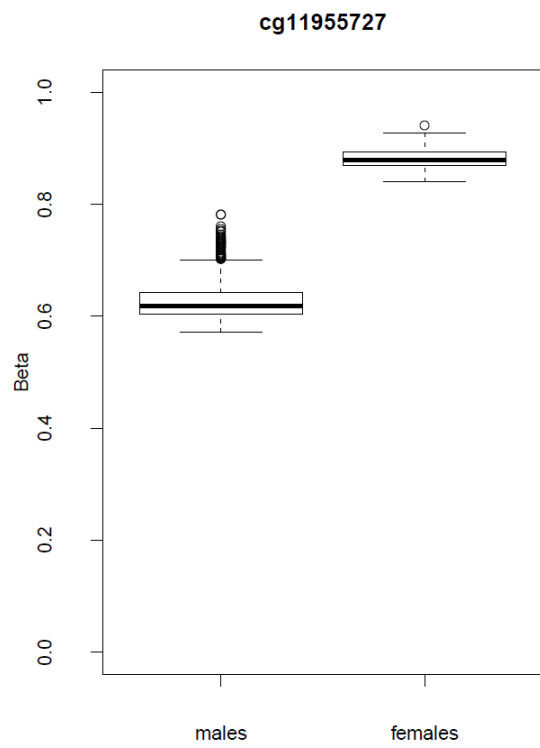
(A) Supplementary Files

Four excel files are made publically available at: <http://www.sickkids.ca/Research/Weksberg-Lab/Publications/index.html>

1. List of cross-reactive probes (as well as list of non-specific ch probes)
2. List of polymorphic CpGs (and for Infinium I, SNPs at site of single base extension) targeted by the array (as well as list of SNPs underlying probe hybridization sequences)
3. Formatted BLAT output of cross-reactive sites with at least 48bp matched to the probe target sequences
4. Formatted BLAT output of cross-reactive sites with 47bp matched to the probe target sequences

(B) Supplemental Figures





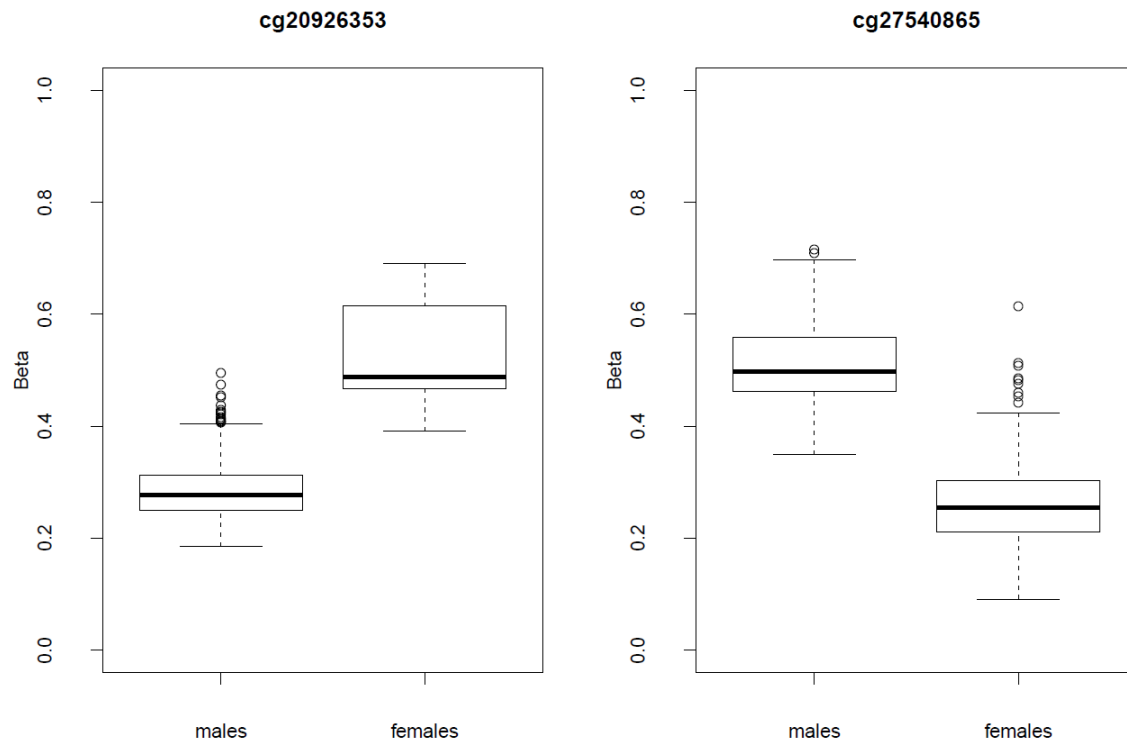
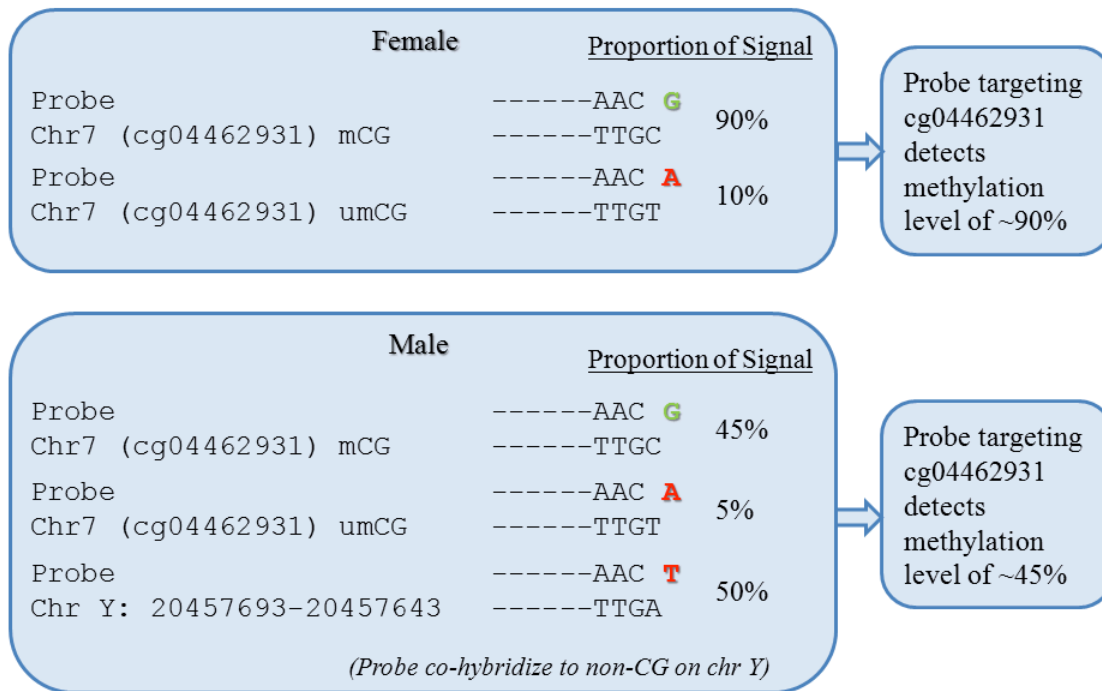


Figure S1. Ten autosomal CpG sites demonstrate significant sex methylation differences as the result of cross-reactive probes co-hybridizing to both autosomal and sex chromosomes. Probes targeting cg00167275, cg02015053, cg12073911, cg16218221, cg20926353 co-hybridize to Chr. X, while probes targeting cg00500229, cg04462931, cg11955727, cg18382982, cg27540865 co-hybridize to Chr. Y. Whether or not the methylation is greater in males than females or vice versa does not seem to depend on which sex chromosomes the cross-reactive probes co-hybridize to.

Schematic representation of cross-reactive probes

Probe targeting cg04462931 (as shown in Figure 2) co-hybridizes to chr Y (50bp match)



Schematic representation of polymorphic CpG

Probe targets cg05455372 - a polymorphic CpG cytosine (rs2863984) (as shown in Figure 2)

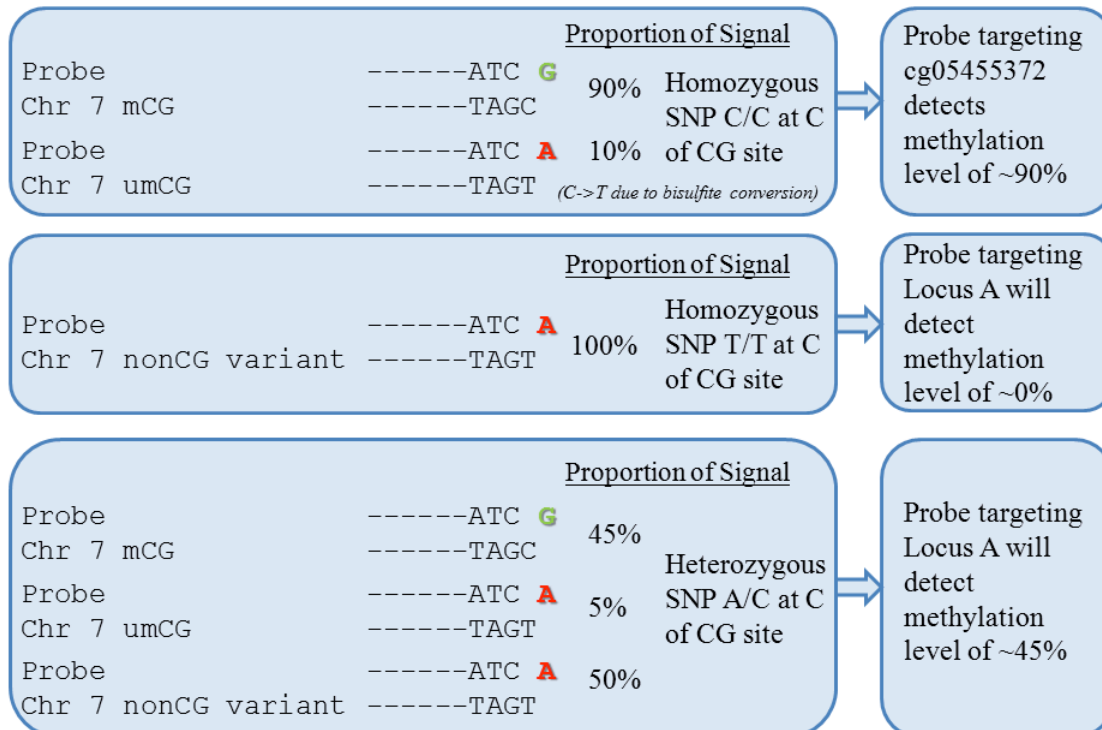
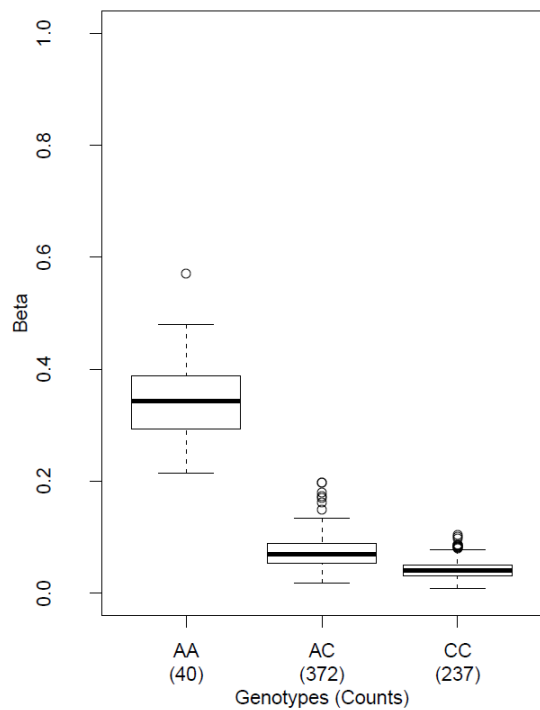
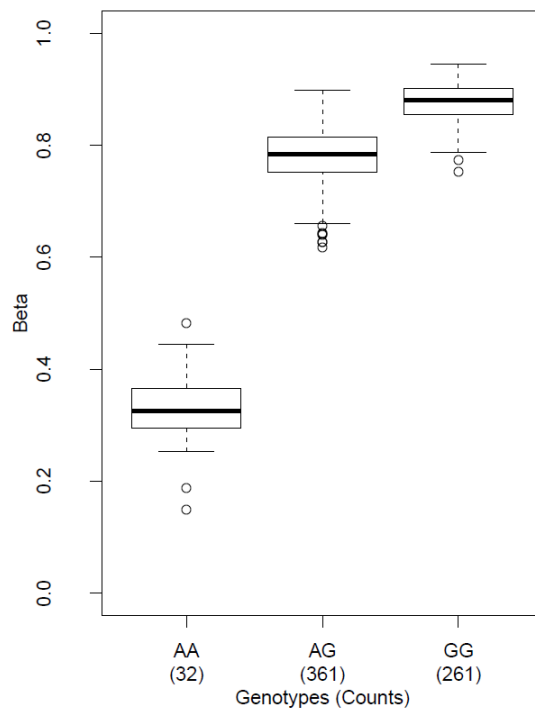


Figure S2. A) Schematic representation of cross-reactive probe and B) of polymorphic CpG. Probe targeting cg04462931 co-hybridize to a cross-reactive site on the chr Y (50bp match), and such cross-reactive site has non-CG (A instead of C) being detected as fully unmethylated locus by the microarray. Probe targeting cg04462931 also detects non-CG as fully unmethylated locus when the SNP allele has A at the C position of CG. This results in methylation profiles reflect the underlying genotype.

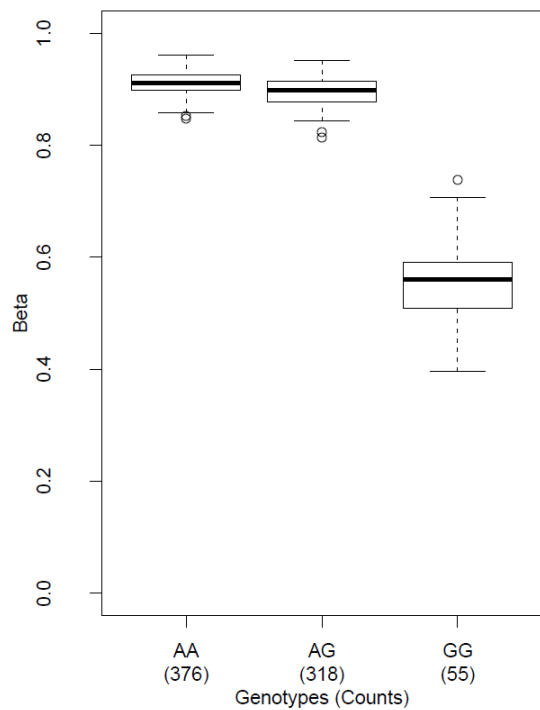
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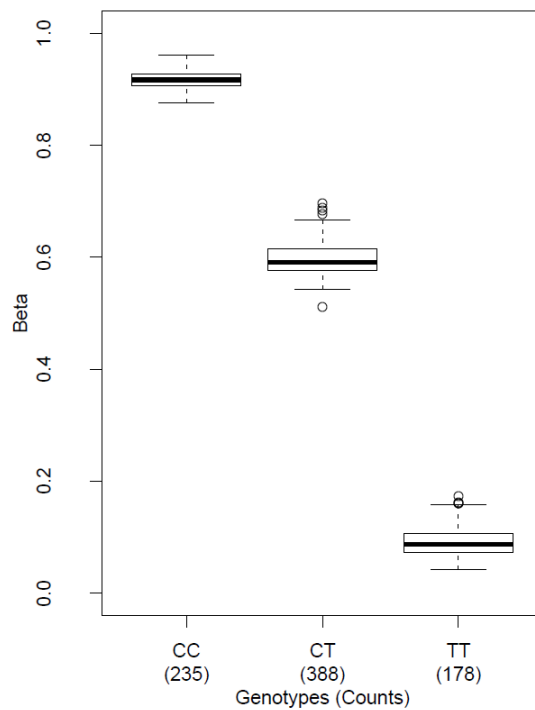
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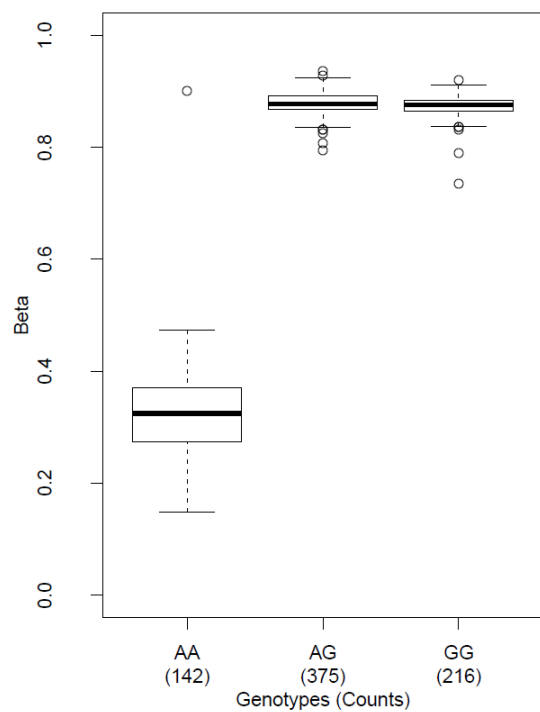
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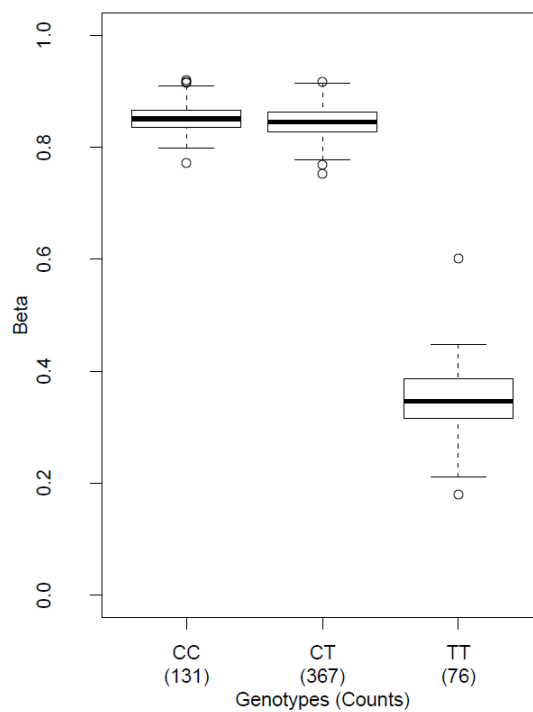
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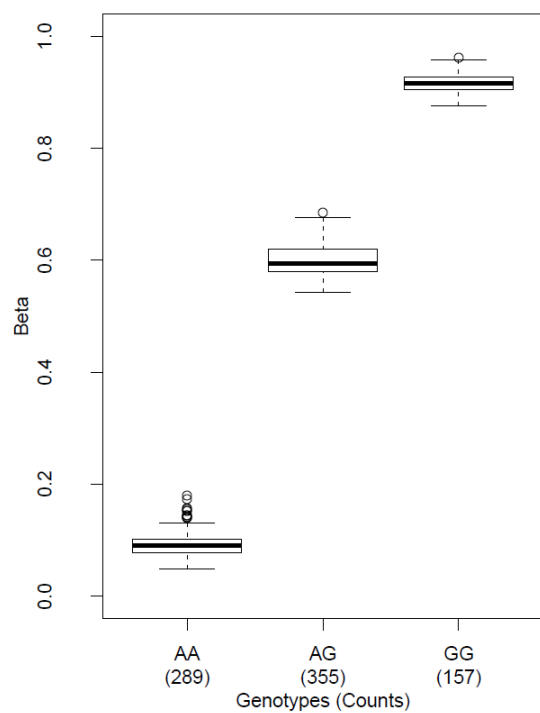
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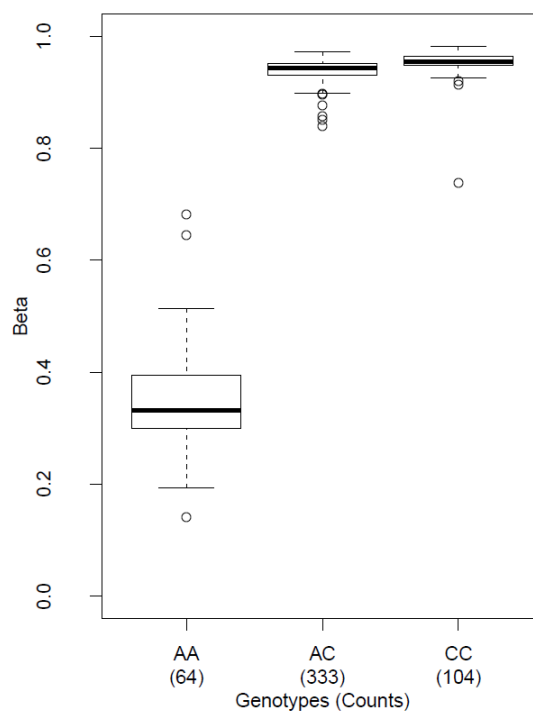
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cg08238375/rs4705795



cg02262167/rs1163860



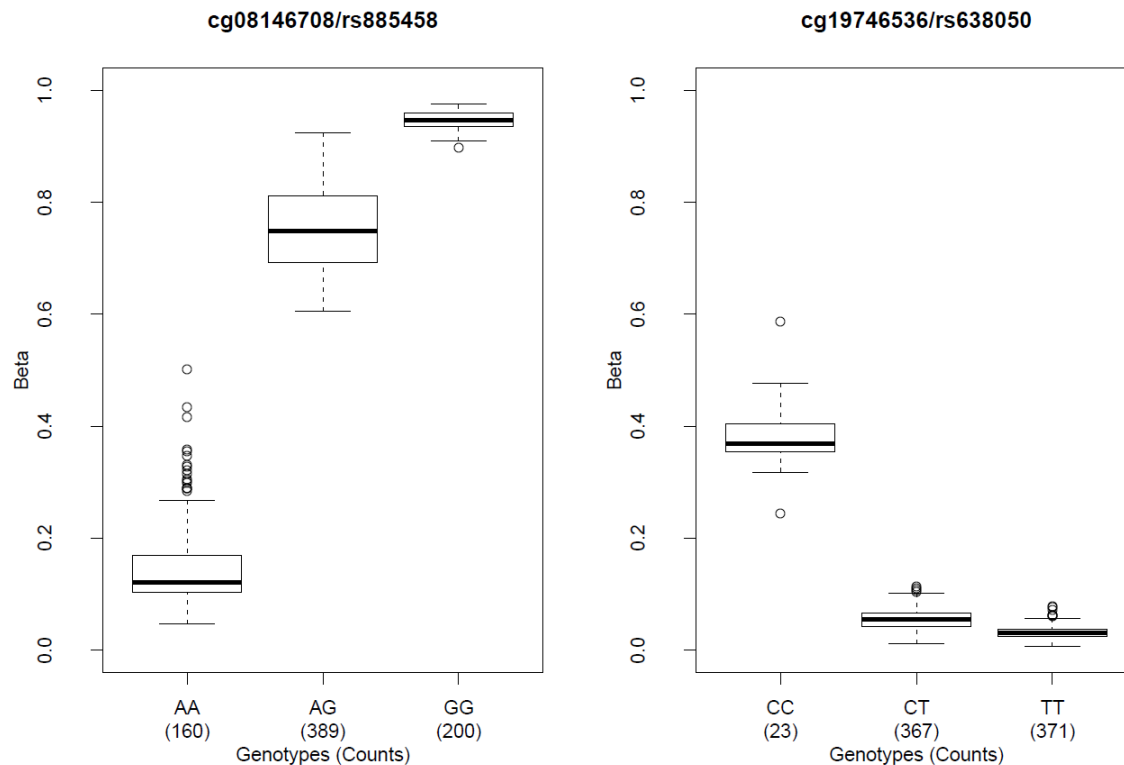
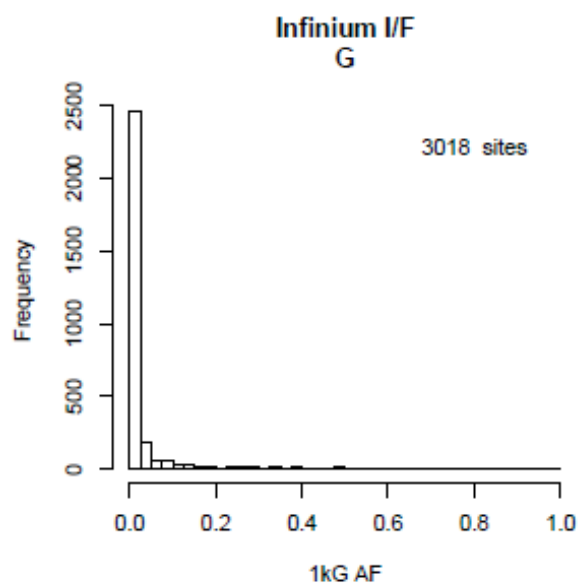
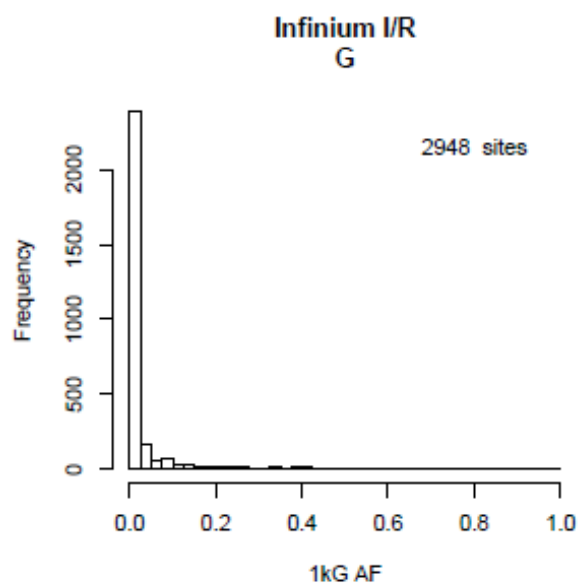
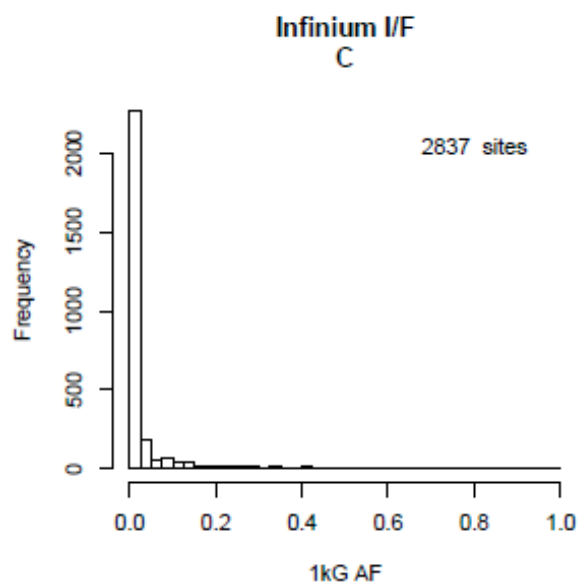
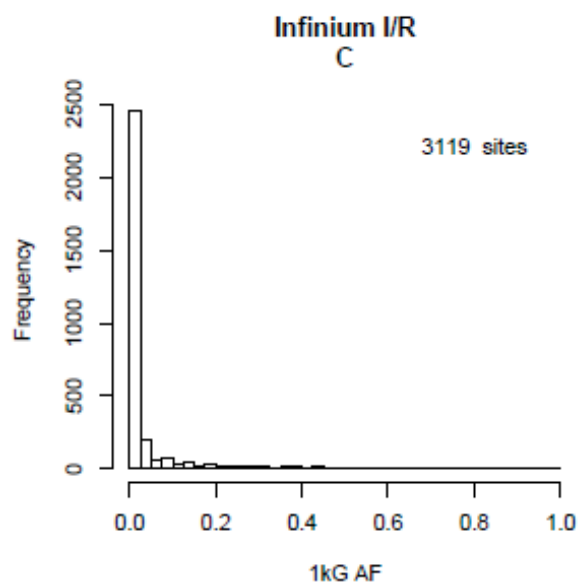
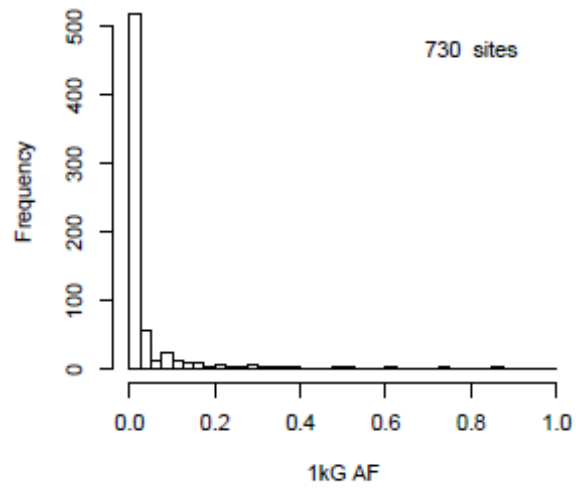


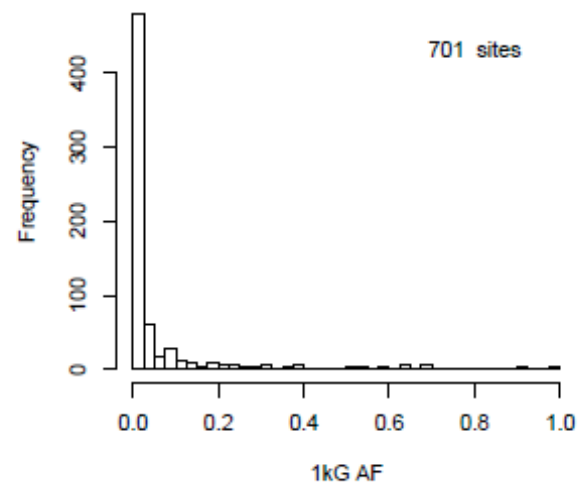
Figure S3. Ten polymorphic CpG sites demonstrate methylation profiles reflective of the underlying SNP genotypes. For cg25613667 (Infinium I, CpG at the same strand as SNP), cg19949776 (Infinium II, same strand), cg08238375 (Infinium II, opposite strand), and cg08146708 (Infinium I, opposite strand), SNPs are located at the C position of CpGs. For cg01270299 (Infinium I, same strand), cg08458132 (Infinium II, same strand), cg05276972 (Infinium II, opposite strand), and cg02262167 (Infinium I, opposite strand), SNPs are located at the G position of CpGs. For cg06652011 (Infinium I, same strand) and cg19746536 (Infinium I, same strand), SNPs are located at the base before the CpGs (i.e. position of single base extension for Infinium I assay). Some methylation profiles may be counterintuitive, for example cg25613667: the AA genotype should render CpG to CA dinucleotides and thus should have no methylation. However, the low signal intensity or noise generated by the probe with no target would result in 1) methylation levels failed to pass detection p-value cutoff (and thus low call counts; for cg25613667 only 44 AA compared to 372 AC or 237 CC) and 2) intermediate methylation levels (since there will be equal amount of signal noise detected by both the methylated and unmethylated probes) barely pass the detection p-value cutoff.



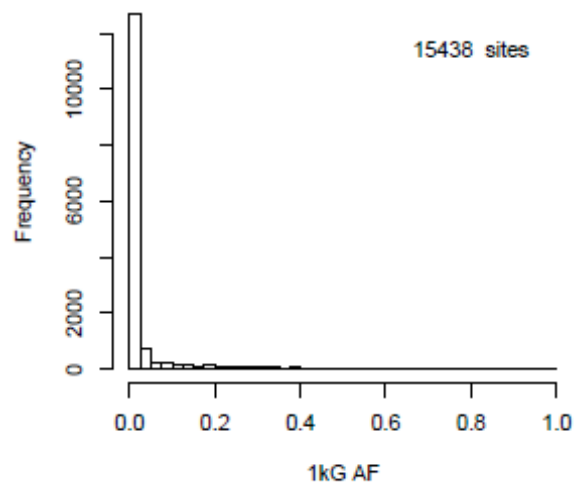
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SBE**



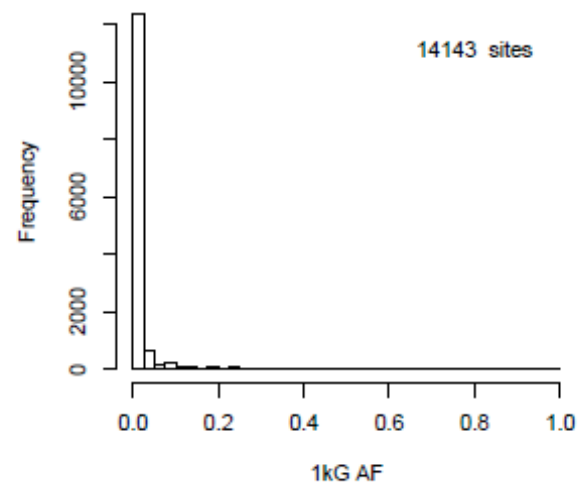
**Infinium I/F
SBE**



**Infinium II/R
C**



**Infinium II/F
C**



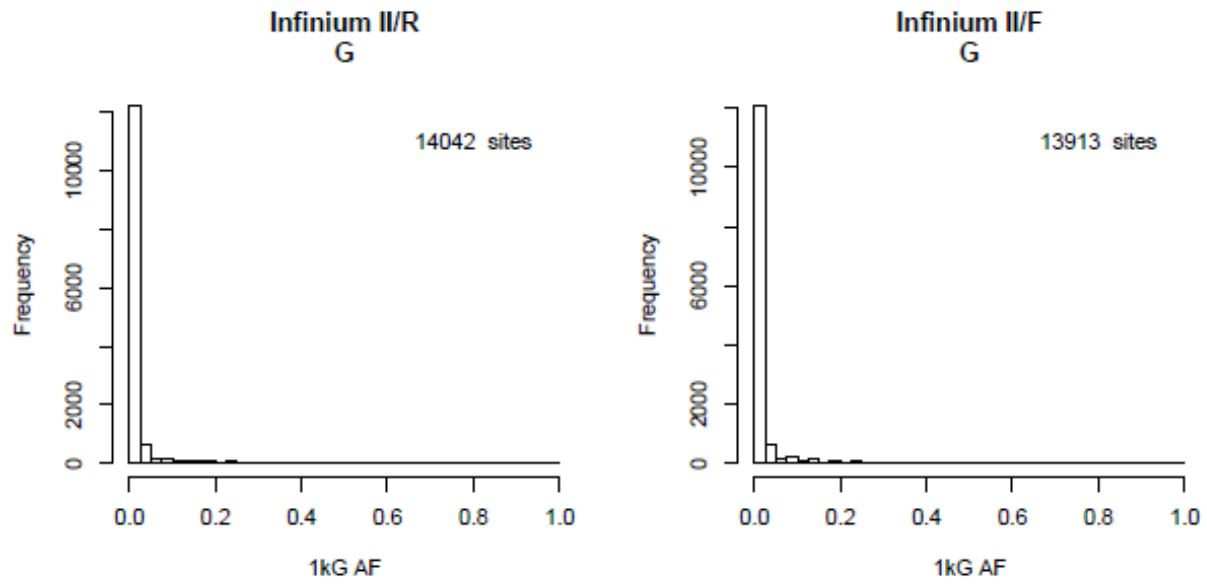


Figure S4. Non-reference allele frequency of SNPs overlapping CpGs (i.e. polymorphic SNPs) and the base before CpGs (i.e. position of single base extension for Infinium I assay). Graphs are stratified based on the assay type (Infinium I or II), the targeted strand (Forward or Reverse) and the polymorphic base (Cytosine, Guanine or position of single base extension [SBE]). The number of polymorphic sites is indicated.